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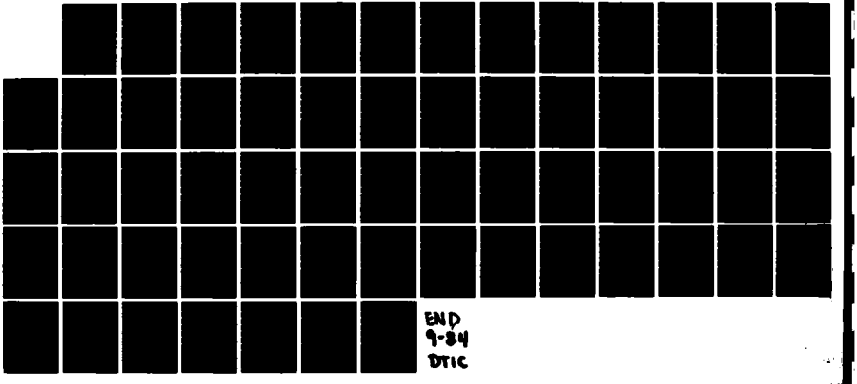

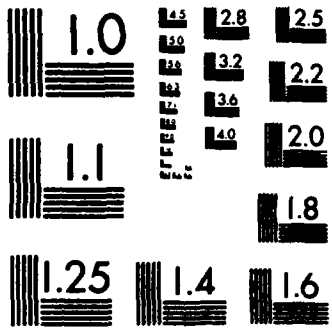

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Annual Report

Metabolic Studies on WR-158,122 in Bile Duct Cannulated Rats and Monkeys

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Section 1: Blood levels, excretion, and tissue distribution of WR-158,122 ^{14}C were studied in one monkey, 7 bile duct cannulated rats, 8 bile duct ligated rats and 2 control rats. The monkey necropsied at 12 hours after dosing excreted 1.8% of the dose in urine and none in feces. It retained 93.6% of the dose in the gastrointestinal tract plus contents. The stomach contained 77.1 percent of the dose, and as a result there was very little drug and/or its metabolites in any other tissue. Blood and plasma peak level

Abstract (continued):

occurred at 4 hours and plasma levels were higher than those in whole blood. Blood levels of bile duct ligated rats were appreciably higher than the blood levels of bile duct cannulated rats, but the blood curves peaked at the same time and followed the same general pattern. Bile duct cannulated rats excreted 11.6-56.9 percent of WR-158,122 dose (as ^{14}C) in the bile. These rats generally excreted very little drug in the urine (4.5%). Bile duct ligated rats excreted 13.5-36.3 percent of drug (as ^{14}C) in the urine. Feces are the main excretion route for WR-158,122 (as ^{14}C) in both bile duct cannulated and bile duct ligated rats accounting for 32.3 percent to 84.5 percent of the dose in the bile duct cannulated rats and from 49.6 to 81.3 percent in the bile duct ligated animals. Excretion of ^{14}C in both urine and feces was essentially complete at 48 hours. In both bile duct cannulated and bile duct ligated rats there is very little residual ^{14}C at 72 hours in liver, (heart, lungs, spleen, kidneys as a pool), gastrointestinal tract and contents, or carcass. Thus, in the rat it appears that WR-158,122 is rapidly, but incompletely absorbed, transported to the liver and excreted in the bile.

Section 2: A 4 kg female rhesus monkey with a bile duct cannula was administered two single oral doses of ^{14}C-labeled WR-158,122. The first dose was given 7 days after initial surgery and the second dose 9 days later. Part (approximately 50%) of the first dose was lost by vomiting but the entire second oral dose was retained. Following the first treatment total excretion of drug in the urine (13.1%) and feces (32.8%) was equivalent (as ^{14}C) to 45.9% of the dose, not corrected for loss from vomiting. An additional 4.12% was excreted in the bile of which about 75% was returned to the animal. Following the second 5 mg/kg oral dose, excretion (as ^{14}C) in the urine (21.5%) and feces (72.8%) accounted for 94.3% of the dose. An additional 9.4% was excreted in the bile and again about 75% was returned. These data indicate that at least in this monkey, WR-158,122 (as ^{14}C) is moderately absorbed and to a limited degree excreted via the bile.

Section 3: Urine and bile samples from control (untreated) rats and rhesus monkeys as well as from bile duct ligated (BiDuLi) rats and bile duct cannulated (BiDuCa) rats and monkeys treated with WR-158,122-^{14}C have been extracted with a series of organic solvents in an attempt to define the profile of biliary and urinary metabolites. Extraction of a urine sample from a BiDuCa monkey demonstrated that when the urine was saturated with KBr very polar solvents such as n-propanol (NPRO), pyridine, dimethylformamide or methanol formed one phase (with some salt) and the solvent phase contained all or nearly all of the ^{14}C activity. Solvents forming 2 phases such as ethylene dichloride: 2-ethylhexanol (8:2, EDC-2EH), ethyl acetate, acetonitrile (ACN) or tetrahydrofuran (THF) extracted increasing amounts of ^{14}C-containing metabolites ranging from 8.6% to 66.2%. NPRO, EDC-2EH, ACN and THF extracted all or nearly all of the ^{14}C activity from control simian urine and bile and from control rat urine. EDC-2EH, ACN and THF extracted increasing amounts of ^{14}C from a 72 hr monkey treatment bile but even THF extracted only 27% of the total ^{14}C content. The total parent drug in this sample was 3% or less. The ^{14}C activity in a 12 hr treatment bile sample from a BiDuCa rat gave an extraction profile not much different from that obtained with simian treatment bile; less than 4% of the activity comprised parent drug. When one compares 24 hr urine samples from BiDuCa and BiDuLi rats one finds that the only significant difference in the profiles is in the amount of ^{14}C activity extracted by EDC-2EH which was about 14% for the BiDuLi rat and 23% for the BiDuCa animal. These data provide

Abstract (continued)

almost indisputable evidence that WR-158,122 is absorbed and metabolized in the albino rat and rhesus monkey and the proportion of extractable ^{14}C activity is greater in the urine than in the bile. This is especially true for the monkey.

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Block 19 - Key Words

sulfaquinazoline
WR-158,122
2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline
rhesus monkey
rat
labeled drug, ^{14}C
bile duct cannulation
bile duct ligation
blood levels
biliary excretion
urinary excretion
fecal excretion
tissue distribution
organic solvent extraction
urine extraction
bile extraction
solubility parameter

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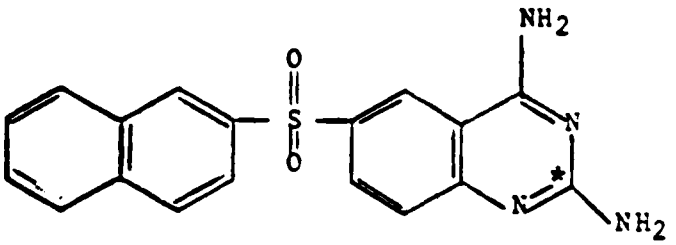
SECTION 1. METABOLIC DISPOSITION OF LABELED WR-158,122 IN BILE DUCT CANNULATED AND BILE DUCT LIGATED RATS

Studies on the metabolic fate of WR-158,122 [2,4-diamino-6-(2 naphthylsulfonyl)-quinazoline-2-^{14}C], the antifolate antimalarial compound shown below



*^{14}C

have been carried out in one rhesus monkey, 7 bile duct cannulated rats, 8 bile duct ligated rats and 2 control rats. Data on blood levels, excretion and tissue distribution are detailed in this report.

1.2 MATERIALS AND METHODS

Compounds. WR-158,122 [2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline-2-^{14}C], empirical formula $C_{18}H_{14}SO_2N_4$, mol. wt. 350, was supplied by Walter Reed unlabeled as Bottle AY 65859. The ^{14}C-labeled preparation was synthesized by Research Triangle Institute and dated 1/8/79. Lot No. 2572-110, dated 8/20/79, has a specific activity of 69 µCi/mg or 24 mCi/mmole. The radiochemical purity in a number of TLC systems was > 98%.

Treatment Suspension. The treatment suspension was prepared by grinding intimately 307.13 mg cold drug and 3.08 mg of ^{14}C labeled drug in a glass mortar with glass pestle with addition of small amounts of diluent (0.2% methylcellulose and 0.4% Tween 80 in distilled water) until a smooth suspension was achieved. The suspension was decanted into a tared round-bottom polycarbonate 200 ml centrifuge bottle containing glass beads and diluted to 124.08 g. It contained 2.50 mg WR-158,122/ml and 1.86 µCi/ml. The suspension was stored at 4°C. Assay of the suspension gave the following results:

4,136,000 dpm/ml
1654 dpm/µg
1.86 µCi/ml

Analytical Procedures

1. Blood. 0.2 or 0.3 ml of blood is digested with 0.5 ml of 0.5 N NaOH at 70°C. After 2 hours, 0.5 ml of t-butyl hydrogen peroxide (TBHP) is added and the sample is incubated for an additional 2 hours. After cooling the cocktail (15 ml of 4% DTN) is added followed by immediate vigorous shaking. After 2 hours cooling vials are counted in a Packard Tri Carb. All samples are corrected for quenching and other effects by internal standardization.

DTN Media

PPO Concentrate	Final Media
1 liter toluene	150 ml PPO concentrate is added to 3 l of dioxane
100 g PPO	Add 300 g naphthalene
2.5 g DiMePOPOP	Add 3.5 g BHT*
	Add 140 g Cab-O-Sil® (ca 4%)

* 2,6-Di-tert-butyl-p-cresol

2. Plasma. 0.5 ml of plasma is digested using the same procedure (eliminating the bleaching step) described for blood above.

3. Bile. Bile is counted exactly like blood except that the sample is usually 0.5 ml.

4. Urine. 0.5 ml samples of urine can usually be counted directly, but some highly-colored samples may require bleaching.

5. Feces. Feces are digested in appropriately sized jars with 10 volumes of 0.5 N NaOH for 48 hours at 70°C. Replicate 0.5 ml samples of the warm but not hot digest are bleached with 0.25 ml of TBHP for 2 hours and completed as above.

6. Tissues. Tissue samples in appropriately sized jars are digested with 4 volumes of 0.5 N NaOH at 70°C for 24 hours. Gastrointestinal tract and contents may require 48 hours. Replicate 0.5 ml or larger samples are bleached with TBHP 2 hours and counted as described above.

7. Carcass. Carcass is cut into several sections and a weight of H_2O equal to the carcass weight is added and 10 ml of 19 N NaOH are added for each 100 g of combined carcass and water. The digest after 24 hours is bleached and counted in the same way as the tissues described above. The bones do not digest in alkali but can be prepared for counting by dissolving them in an appropriate volume of 25% HNO_3 heated to about 90°C for several hours.

0.5 ml samples of this digest are counted in DTN-Cab-O-Sil. We found that for WR-158,122, the bones contained negligible amounts of ^{14}C activity.*

Monkey. One female rhesus monkey was placed in a chair approximately 24 hours before treatment using a small dose of Ketaset®. Water was available ad libitum. Control urine (216 ml) and control feces (23.8 g) were collected. The chair facilitated the collection of repeated blood samples as well as the quantitative collection of urine (in an iced container) and feces. The drug was administered by soft oral catheter as a single dose of 5 mg/kg. Blood samples were collected from the right or left antecubital vein at 2, 4, 6, 8, and 12 hours. At 12 hours the animal was given an anesthetic dose of Nembutal® and tissues were collected for distribution studies.

Rats. Sprague-Dawley-derived male rats weighing 245-376 g (bile duct ligated) and 220 to 346 g (bile duct cannulated) at time of surgery were used. The bile duct ligated rats were housed in metabolism cages. The bile duct cannulated rats were placed in wire restrainers attached to the metal cover of a plastic cage. Food and water were supplied ad libitum 4 hours after dosing. A bottle equipped with a funnel and a wire separator was positioned to collect all urine and feces. A tared vial was secured to the cage to collect bile. Blood samples were obtained at 2, 4, 6, 8, 12, 24, 48, and 72 hours from the tail vein in heparinized 200 µl pipettes. Bile, urine and feces were usually collected at 6, 12, 24, 48 and 72 hours. At 72 hours the rats were necropsied using an anesthetic dose of Nembutal®. Tissues assayed for ^{14}C included 1) liver; 2) heart, lungs, spleen and kidneys as a pool; 3) gastrointestinal tract and contents and 4) carcass.

*We are investigating other methods of measuring ^{14}C content of bone.

Bile Duct Cannulation Procedure. The rats are fasted overnight and the following morning they are anesthetized with sodium pentobarbital (50 mg/kg i.p.). The abdomen is shaved and a transverse incision through the skin is made caudal to the last rib. The muscle layer is lifted, using forceps, and cut with blunt tip scissors being careful to avoid cutting the underlying tissues. The liver is retracted cranially using a warm saline-moistened gauze pad.

The area where the bile duct is cannulated is just caudal to the liver and superficial to the portal vein. Two ligatures are placed around the bile duct, the first caudal to the bifurcation of the bile duct and the second 5 mm caudal to the first ligature. The second ligature is tied causing the bile to build up in the bile duct. A small incision is made in the bile duct between the ligatures using a small scissors.

A polyethylene cannula (Clay-Adams PE 50), with one end cut at a 45 degree angle, is inserted into the bile duct and secured with the first and second ligature.

The cannula is exteriorized between the skin and muscle layer of the medial aspect of the left hind leg.

The muscle and skin layer of the abdomen are sutured separately and the skin is sealed with collodion.

When surgery is completed, the animal is placed in a stainless steel wire restrainer cage with the left hind leg secured to the restrainer using cotton cord with a ligature through the skin at the Achilles tendon (adapted from Robert E. Smyth, personal communication, 1977).

Bile-Duct Ligation Procedure. The rats are fasted overnight and the following morning they are anesthetized with sodium pentobarbital (50 mg/kg i.p.).

The abdomen is shaved and a transverse incision through the skin is made caudal to the last rib approximately 2½ cm in length. The muscle layer is lifted, using forceps, and cut with blunt tip scissors being careful to avoid cutting the underlying tissues. The liver is retracted cranially using a warm saline-moistened gauze pad.

The area where the bile duct is ligated is just caudal to the liver and superficial to the portal vein. Two ligatures of 4-0 silk are placed around the bile duct, the first caudal to the bifurcation of the bile duct and the second 5 mm caudal to the first ligature. Both ligatures are tied and the section of duct between the ligatures is cut with an iris scissors.

The muscle and skin layer of the abdomen are sutured separately and the skin is sealed with collodion.

1.3 RESULTS

A. Monkey. Blood Levels, Excretion and Tissue Distribution

Data on whole blood and plasma levels of WR-158,122 (as ^{14}C) were as follows:

Hours Post Dose	µg/g as ^{14}C	
	Blood	Plasma
2	0.18	0.25
4	0.43	0.62
6	0.40	0.58
8	0.33	0.49
12	0.36	0.46

Peak levels of radioactivity appeared in the blood and plasma at 4 hours. The plasma levels always exceeded the whole blood concentrations, findings which corroborate data on blood levels in rhesus monkeys submitted in Interim Report No. 40-1 on Contract No. DADA 17-67-C-7065 (March 16, 1972).

This monkey excreted 1.8% of the dose (as ^{14}C) in urine in 12 hours (see Table 1). No feces were excreted but the gastrointestinal tract and contents contained 93.6 percent of the dose (as ^{14}C) of which 77.1 percent was recovered in the stomach. There was very little localization of the compound (or its metabolites) in any tissue (Table 2), but this was probably due to the large amount of drug (^{14}C) still in the stomach.

B. Bile Duct Cannulated Rats.

Blood Levels. The peak for blood levels varied widely ranging from 0.21 to 0.87 µg/g (as ^{14}C) (see Table 3 and Figure 1). Two rats had peak levels at 4 hours, four at 12 hours and one at 24. One rat (BC-6) showed a double peak at 12 hours and 48 hours.

Biliary Excretion. Total recovery of WR-158,122 and metabolites (as ^{14}C) in the bile by 72 hours ranged from 11.4 to 56.9 percent of dose (see Table 4 and Figure 2). This range is narrowed somewhat if BC-6 is eliminated. This animal is atypical in that it retained much more drug in the gut at necropsy time and eliminated much less in the feces than the other bile duct cannulated rats. It also had an atypical double-peaked blood level curve. Peak levels of drug (as ^{14}C) in the bile occurred at 12 hours in two rats, at 24 hours in three rats, and at 48 hours in two animals. The peak for BC-1 might have occurred at a different time if bile flow had not been temporarily interrupted between 12 and 24 hours. In rat BC-2, the cannula became inoperative some time after the 48 hour collection and this accounts for the very small bile output at 72 hours. In rat BC-3 the cannula came out of the collection vial after 48 hours excluding the opportunity of collecting a 72 hour sample.

Urine Excretion. Total recovery of WR-158,122 (as ^{14}C) in the urine of bile duct cannulated rats ranged from 0.4 to 15.6 percent of dose and is

essentially complete at 48 hours (see Table 4). As one would expect the range narrows to 0.4 to 8.5 percent if the data for BC-1 (a rat with temporary biliary stasis at 12-24 hours) is eliminated. The peak levels of drug as ^{14}C in the urine occurred at 24 hours in 4 rats and at 48 hours in the remaining animals.

Fecal Excretion. Excretion of drug and metabolite(s) in the feces was essentially complete in 48 hours. Total recovery in feces for the seven bile-duct cannulated rats ranged from 32.3-84.5 percent of dose (see Table 4). In rat BC-1, 50 percent of the dose was excreted in the 12-hour feces sample, but peak fecal excretion usually occurred at 24 hours.

Total Recovery. Total recovery varied from 87.2 to 114.7 percent of the dose with a mean of 101% (see Table 5). Fecal excretion accounted for an average of 65%, biliary excretion for 27% and urinary output for 4.5%. Except for the terminally atypical rat, BC-6, with apparent megacolon, the remaining radioactivity in all body compartments at 72 hours was usually less than 2%.

C. Bile Duct Ligated Rats.

Blood Levels. As in the bile duct cannulated rats, the peak blood levels varied widely (Table 6 and Figure 1). Three rats showed peaks at 12 hours. one rat at 24 hours and 2 rats at 48 hours. The unexpectedly high value for BL-6 at 48 hours (1.78 µg/g) may be an error due to some unrecognized contamination of the sample. It was not included in the average.

Urine Excretion. Total recovery of WR-158,122 (as ^{14}C) in the urine of bile duct ligated rats ranged from 13.5 to 36.3 percent of dose (Table 7 and Figure 3). Excretion of drug in the urine was essentially complete at 48 hours. Peak urine excretion occurred at 12 hours for one rat, 24

hours for four rats, and at 48 hours for one animal.

Fecal Excretion. Excretion in the feces was essentially complete in 48 hours (see Table 7). Total recovery in feces for the six bile duct ligated rats ranged from 49.6 to 81.3 percent. Peak fecal excretion occurred at 12 hours for one rat, at 24 hours for two rats, and at 48 hours for three rats.

Total Recovery. Total recovery ranged from 82.2 to 98.9 percent of the dose for the six bile ligated rats with a mean of 89.4 percent (see Table 8). It is readily apparent that fecal excretion accounted for most of the drug. In all rats there was very little ^{14}C in the gastrointestinal tract and contents at 72 hours, and this was true for other tissues and carcass as well.

D. Control Rats Sacrificed at 24 Hours

In control rats sacrificed at 24 hours after dosing, 84-94 percent of the dose was recovered in feces (see Table 9). C-2 retained about 5 percent of the dose in the gut and contents and C-1 retained only 1.0 percent. Urinary excretion amounted to 2 to 5 percent and there was very little ^{14}C in the other tissues.

E. Bile Duct Ligated Rats Sacrificed at 24 Hours.

In two bile duct ligated rats sacrificed at 24 hours after dosing 43 to 57 percent of the dose was present in the feces. In both rats 11 percent of the dose was recovered in the urine and they retained 24 to 25 percent of the dose in the gastrointestinal tract and contents. Other organs (liver, heart, lungs, spleen, and kidneys) contained 1.2 to 1.5 percent of the dose and the carcass contained 6.4 and 8.8 percent of the dose as ^{14}C.

1.4 DISCUSSION

The data on the single 12-hour monkey appear to fit with our previous simian data collected at other time periods (see Report 40 of DADA 17-67-C-7065, 3/16/72).

From the data on biliary excretion in bile duct cannulated rats it was concluded that WR-158,122 was partially absorbed and excreted in the bile. Two rats appeared to be atypical. In BC-1 the cannula closed temporarily between 12 and 24 hours and reopened some time between 24 and 48 hours which gave results mimicing a bile duct ligated rat. Rat BC-6 retained feces so heavily in the gut that its condition bordered on megacolon. This rat retained 12.1 percent of the dose in gastrointestinal tract and contents at 72 hours whereas the other bile duct cannulated rats retained less than 1 percent. This rat also excreted the largest amount of drug and/or metabolites in its bile (57%). Rat BC-A was a pilot experiment and the rat was appreciably heavier than the six other bile duct cannulated rats; however, the data on this rat compare very well with the data of the six bile duct cannulated rats run later except for higher blood level data (see Table 3).

When blood levels of bile duct cannulated rats were compared with blood levels of bile duct ligated rats it was apparent that levels for bile duct cannulated rats were appreciably lower than levels for bile duct ligated rats (Figure 1) but the peak blood levels for both groups occurred at 12 hours and the curves followed the same general pattern.

In our earlier work with control rats the peak blood levels of WR-158,122 (as ^{14}C) occurred at 3 hours after dosing (Interim Report No. 40-1) whereas in bile duct cannulated and bile duct ligated rats the peak levels usually occurred at 12 hours or later.

As one might anticipate bile duct ligated rats excreted much more drug in the urine (20%) than bile duct cannulated rats (4.5%). Fecal excretion of ^{14}C in bile duct cannulated and bile duct ligated rats was comparable. The one rat, BC-6, with low fecal excretion (32.3 percent of dose) had 56.9 percent of its dose in the bile and 12.1 percent of its dose in gastrointestinal tract plus contents. The one rat in the bile duct ligated series (BL-5) with somewhat low fecal excretion (49.6 percent of dose) excreted the largest amount (36.3%) in the urine.

Except for BC-6 there was very little residual ^{14}C in gastrointestinal tract plus contents in either the bile duct cannulated or the bile duct ligated animals. It is interesting that the two bile duct ligated rats sacrificed at 24 hours retained about 25 percent of the dose in the gut. Control rats on the other hand retained only 1-5 percent of the dose in gastrointestinal tract and contents at 24 hours.

At 72 hours both bile duct cannulated and bile duct ligated rats retained less than 1.0 percent of the dose in the other tissues which included liver, heart, lungs, spleen and kidneys.

The mean liver wt./body wt. ratio for bile duct cannulated rats was 0.039 ± 0.004 and that for bile duct ligated animals was 0.052 ± 0.002. Thus the mean liver weight for bile duct cannulated rats was 9.3 g and for bile-duct ligated rats was 14.6 g. The liver of bile duct cannulated rats contained 0.4% of the dose of ^{14}C on the average, whereas bile duct ligated rats with larger livers contained only about one-third as much ^{14}C.

Carcass retained very little of the dose at 72 hours, usually less than 1%. In the two bile duct ligated rats sacrificed at 24 hours the carcass retained 6 to 9 percent of the dose.

1.5 SUMMARY

1. Blood levels, excretion, and tissue distribution of WR-158,122 ^{14}C were studied in one monkey, 7 bile duct cannulated rats, 8 bile duct ligated rats and 2 control rats.

2. The monkey necropsied at 12 hours after dosing excreted 1.8% of the dose in urine and none in feces. It retained 93.6% of the dose in the gastrointestinal tract plus contents. The stomach contained 77.1 percent of the dose, and as a result there was very little drug and/or its metabolites in any other tissue. Blood and plasma peak level occurred at 4 hours and plasma levels were higher than those in whole blood.

3. Blood levels of bile duct ligated rats were appreciably higher than the blood levels of bile duct cannulated rats, but the blood curves peaked at the same time and followed the same general pattern.

4. Bile duct cannulated rats excreted 11.6-56.9 percent of WR-158, 122 dose (as ^{14}C) in the bile. These rats generally excreted very little drug in the urine (4.5%).

5. Bile duct ligated rats excreted 13.5-36.3 percent of drug (as ^{14}C) in the urine.

6. Feces are the main excretion route for WR-158,122 (as ^{14}C) in both bile duct cannulated and bile duct ligated rats accounting for 32.3 percent to 84.5 percent of the dose in the bile duct cannulated rats and from 49.6 to 81.3 percent in the bile duct ligated animals.

7. Excretion of ^{14}C in both urine and feces was essentially complete at 48 hours.

8. In both bile duct cannulated and bile duct ligated rats there is very little residual ^{14}C at 72 hours in liver, (heart, lungs, spleen,

kidneys as a pool), gastrointestinal tract and contents, or carcass.

9. Thus, in the rat it appears that WR-158,122 is rapidly, but incompletely absorbed, transported to the liver and excreted in the bile.

Table 1.1

Recovery of WR-158,122 from a Rhesus Monkey

Single Oral Dose of 5 mg/kg

	Percent of Dose Recovered as ^{14}C 12 Hours Post Dose
Urine	1.8
Feces	N.S.*
Gastrointestinal Tract & Contents	93.6
Other Tissues	2.0
Bile**	0.02 (1.36 g)
TOTAL	97.4

* No Sample

** The figure in parentheses represents the weight of bile in grams recovered from the gall bladder at time of necropsy.

Table 1.2

Tissue Distribution of WR-158,122 in a Rhesus Monkey

Single Oral Dose of 5 mg/kg

	Recovery (as ^{14}C) in Percent of Dose and µg/g 12 Hours Post Dose	
Tissue	Percent of Dose per Tissue	µg/g
Stomach*	77.1	58.2
Small Intestine*	0.76	2.8
Cecum*	2.4	13.4
Large Intestine*	13.3	17.8
Liver	0.43	0.9
Gall Bladder	<0.01	1.2
Bile	0.02	4.4
Lungs	0.10	0.5
Heart	0.07	1.3
Pancreas	0.04	0.8
Spleen	0.01	0.5
Adrenals	<0.01	0.5
Kidneys	0.13	1.0
Urinary Bladder	0.10	2.7
Muscle, Skeletal+	0.49	0.1
Whole Blood++	0.65	0.4
TOTAL	95.6	

* Tissue plus contents

+ Based on skeletal muscle weight equals 20% of necropsy body weight

++ Based on blood volume equivalent to 9% of necropsy body weight

Table 1.3

Blood Levels of WR158,122 in Bile Duct Cannulated Rats

Single Oral Dose 10 mg/kg

	µg/g as ^{14}C						
Hours Post Dose	BC-1	BC-2	BC-3	BC-4	BC-5	BC-6	BC-A*
1							0.57
2	0.17	0.04	0.12	0.10	0.07	0.06	0.69
4	0.32	0.05	0.21	0.17	0.11	0.12	0.81
6	0.52	0.09	0.20	0.28	0.21	0.12	0.72
8	0.67	0.09	0.20	0.28	0.28	0.23	0.55
12	0.87	0.14	0.17	0.37	0.67	0.34	0.32
24	0.75	0.29	0.15	0.15	0.56	0.28	0.11
48	0.25	0.05	0.04	0.05	0.20	0.34	0.03
72	0.06	0.04	0.01	0.01	0.02	0.11	0.02
Hct (Terminal)	39	41	50	45	46	53	46

* BC-A weighed 480 g; the other rats ranged from 220 to 346 g.

Table 1.4

Excretion of WR-158,122 in Bile Duct Cannulated Rats

Single Oral Dose 10 mg/kg

Sample	Hours Post Dose	Percent Dose Recovered as ^{14}C						
		BC-1	BC-2	BC-3	BC-4	BC-5	BC-6	BC-A
Bile (g)	6	I.S.*	I.S.	6.8 (5.6)	4.4 (5.2)	1.3 (2.5)	2.4 (4.6)	I.S.
	12	11.8 (7.6)	4.8 (7.2)	7.5 (5.4)	8.5 (4.9)	4.2 (2.0)	8.3 (4.7)	8.9 (5.3)
	24	0.08 (5.0)	11.4 (7.4)	8.8 (9.7)	11.1 (9.0)	5.5 (6.9)	17.7 (9.2)	1.8 (7.3)
	48	0.17 (4.4)	21.2 (13.1)	5.6 (14.1)	3.4 (13.5)	3.4 (7.6)	23.2 (14.7)	0.5 (15.0)
	72	0.41 (23.6)	0.09 (1.1)	N.S.**	0.25 (12.5)	0.5 (21.1)	5.3 (14.0)	0.2 (10.9)
TOTAL		12.5	37.5	28.7	27.6	14.9	56.9	11.4
Urine (ml)	6	I.S.	I.S.	0.24 (5.6)	0.03 (0.8)	0.03 (3.9)	0.02 (1.1)	I.S.
	12	3.7 (6.0)	0.42 (3.5)	0.5 (12.0)	0.58 (2.4)	1.2 (4.4)	0.21 (1.6)	0.15 (3.0)
	24	9.9 (13.0)	1.6 (7.1)	0.9 (39.0)	1.3 (12.9)	4.6 (19.2)	2.8 (9.0)	N.S.**
	48	1.8 (21.6)	3.7 (15.2)	0.8 (45.5)	0.4 (24.5)	1.5 (25.3)	4.4 (27.0)	0.19 (19.6)
	72	0.24 (23.0)	0.23 (5.5)	0.05 (23.0)	0.04 (14.2)	0.11 (24.0)	1.1 (17.0)	0.04 (24.0)
TOTAL		15.6	6.0	2.5	2.4	7.4	8.5	0.4

Table 1.4 (continued)

Sample	Hours Post Dose	Percent Dose Recovered as ^{14}C						
		BC-1	BC-2	BC-3	BC-4	BC-5	BC-6	BC-A
Feces (g)	6	I.S.	I.S.	0.01 (1.5)	0.02 (4.5)	N.C.***	N.C.	I.S.
	12	49.9 (4.6)	21.3 (3.1)	16.2 (0.9)	0.01 (0.3)	N.C.	N.C.	28.7 (1.6)
	24	21.7 (2.8)	12.3 (6.2)	40.6 (9.3)	58.2 (4.7)	47.1 (2.0)	28.6 (6.0)	43.4 (3.0)
	48	12.7 (8.8)	21.5 (10.1)	5.9 (7.0)	8.6 (5.4)	27.5 (3.3)	1.5 (0.4)	0.03 (1.4)
	72	0.23 (5.3)	1.3 (1.2)	1.4 (3.7)	1.6 (4.7)	0.5 (5.1)	2.2 (0.6)	2.3 (0.02)
TOTAL		84.5	56.4	64.1	68.4	75.1	32.3	74.4

( ) - Number in parenthesis is sample size in g or ml.

* I.S. - Insufficient sample

** N.S. - No sample

*** N.C. - Sample not collected

Table 1.5

Recovery of WR-158,122 from Bile Duct Cannulated Rats

Single Oral Dose of 10 mg/kg

	Percent of Dose Recovered (as ^{14}C) in 72 Hours						
	BC-1	BC-2	BC-3	BC-4	BC-5	BC-6	BC-A*
Urine	15.6	6.0	2.5	2.4	7.4	8.5	0.4
Feces	84.5	56.4	64.1	68.4	75.1	32.3	74.3
Bile	12.5	37.5	28.7	27.6	14.9	56.9	11.6
Gastrointestinal Tract & Contents	0.5	0.6	0.07	0.03	0.02	12.1**	0.23
Other Tissues	0.9	0.7	0.2	0.2	0.4	0.5	0.3
Carcass	0.7	0.6	0.8	0.1	0.2	0.7	0.4
TOTAL	114.7	101.8	96.4	98.7	98.0	111.0	87.2

* BC-A weighed 480 g; the other rats weighed from 220 to 340 g.

** BC-6 abnormal retention of feces bordering on megacolon.

Table 1.6

Blood Levels of WR-158,122 in Bile Duct Ligated Rats
Single Oral Dose 10 mg/kg

Hours Post Dose	µg/g as ^{14}C					
	BL-1	BL-2	BL-3	BL-4	BL-5	BL-6
2	0.25	0.32	0.18	0.10	0.14	0.28
4	0.45	0.57	0.28	0.13	0.34	0.53
6	0.63	0.72	0.53	0.19	0.43	0.59
8	0.81	0.90	0.69	0.34	0.57	0.77
12	0.85	1.12	0.86	0.46	0.70	0.86
24	0.38	0.52	0.40	0.45	1.16	0.81
48	0.19	0.22	0.12	0.50	0.48	1.78
72	0.07	0.06	0.04	0.14	0.21	0.13
Hct (Terminal)	40	39	49	47	45	43

Table 1.7

Excretion of WR-158,122 in Bile Duct Ligated Rats
Single Oral Dose 10 mg/kg

Sample	Hours Post Dose	Percent Dose Recovered as ^{14}C					
		BL-1	BL-2	BL-3	BL-4	BL-5	BL-6
Urine (ml)	6	0.04 (2.0)	0.9 (2.0)	0.37 (1.7)	0.66 (3.1)	0.56 (1.0)	0.03 (1.4)
	12	4.6 (9.3)	4.4 (9.5)	8.2 (4.1)	1.8 (1.5)	2.5 (1.2)	1.9 (1.5)
	24	4.9 (7.4)	6.3 (6.7)	6.1 (10.0)	5.0 (6.5)	15.6 (5.7)	7.5 (8.2)
	48	3.4 (16.2)	3.5 (20.6)	2.4 (11.8)	9.4 (6.6)	14.9 (11.2)	6.3 (12.0)
	72	0.51 (26.5)	0.39 (18.0)	0.25 (12.2)	1.5 (10.4)	2.7 (13.6)	2.4 (32.0)
TOTAL		13.5	15.5	17.3	18.4	36.3	18.1
Feces (g)	6	11.4 (2.9)	15.6 (3.8)	<0.01 (0.24)	<0.01 (0.13)	N.C.**	N.C.
	12	7.3 (0.26)	12.7 (0.68)	49.6 (3.8)	N.S.*	N.C.	N.C.
	24	21.7 (0.49)	37.5 (2.4)	30.0 (4.3)	14.0 (3.8)	11.3 (2.6)	50.5 (4.0)
	48	26.5 (3.5)	8.5 (4.1)	1.6 (4.3)	53.8 (10.3)	34.1 (5.9)	11.8 (4.4)
	72	1.4 (5.1)	0.62 (8.2)	0.14 (4.5)	1.6 (5.8)	4.2 (5.0)	1.0 (3.2)
TOTAL		68.3	74.9	81.3	69.4	49.6	63.3

( ) - Number in parenthesis is sample size in g or ml.

* N.S. - No sample

** N.C. - Sample not collected

Table 1.8

Recovery of WR-158,122 from Bile Duct Ligated Rats
Single Oral Dose of 10 mg/kg

	Percent of Dose Recovered (as ^{14}C) in 72 Hours					
	BL-1	BL-2	BL-3	BL-4	BL-5	BL-6
Urine	13.5	15.5	17.3	18.4	36.3	18.1
Feces	68.3	74.9	81.3	69.4	49.6	63.3
Gastrointestinal Tract & contents	0.11	0.08	0.03	0.22	0.18	0.14
Other tissues	0.15	0.13	0.11	0.32	0.26	0.21
Carcass	0.35	0.52	0.15	0.53	0.58	0.48
TOTAL	82.4	91.1	98.9	88.9	86.9	82.2

Table 1.9

Recovery of WR-158,122 in Bile Duct Ligated Rats and Control Rats (oral 10 mg/kg)

	Percent of Dose Recovered as ^{14}C 24 Hours After Treatment			
	BL-7	BL-8	C-1	C-2
Urine	10.7	11.2	1.6	4.5
Feces	56.7	42.5	93.9	84.0
Gastrointestinal Tract and Contents	24.7	29.6	1.0	4.9
Other Tissues	1.2	1.8	0.1	0.7
Carcass	4.5	6.4	0.1	0.4
TOTAL	97.9	91.5	96.7	94.5

Table 1.10

Recovery of WR158,122 in Bile Duct Ligated and Control Rats (oral 10 mg/kg)

	µg/g Recovered as ^{14}C 24 Hours After Treatment			
	BL-7	BL-8	C-1	C-2
Gastrointestinal Tract and Contents	37.1	51.9	1.4	6.8
Liver	2.4	3.6	0.3	1.0
Lungs	1.0	2.1	<0.1	0.2
Heart	0.6	1.1	<0.1	<0.1
Spleen	0.7	1.2	<0.1	<0.1
Kidneys	2.4	5.0	0.2	0.4
Whole Blood	0.4	0.6	<0.1	<0.1
Carcass	0.2	0.3	<0.1	<0.1

Figure 1.1

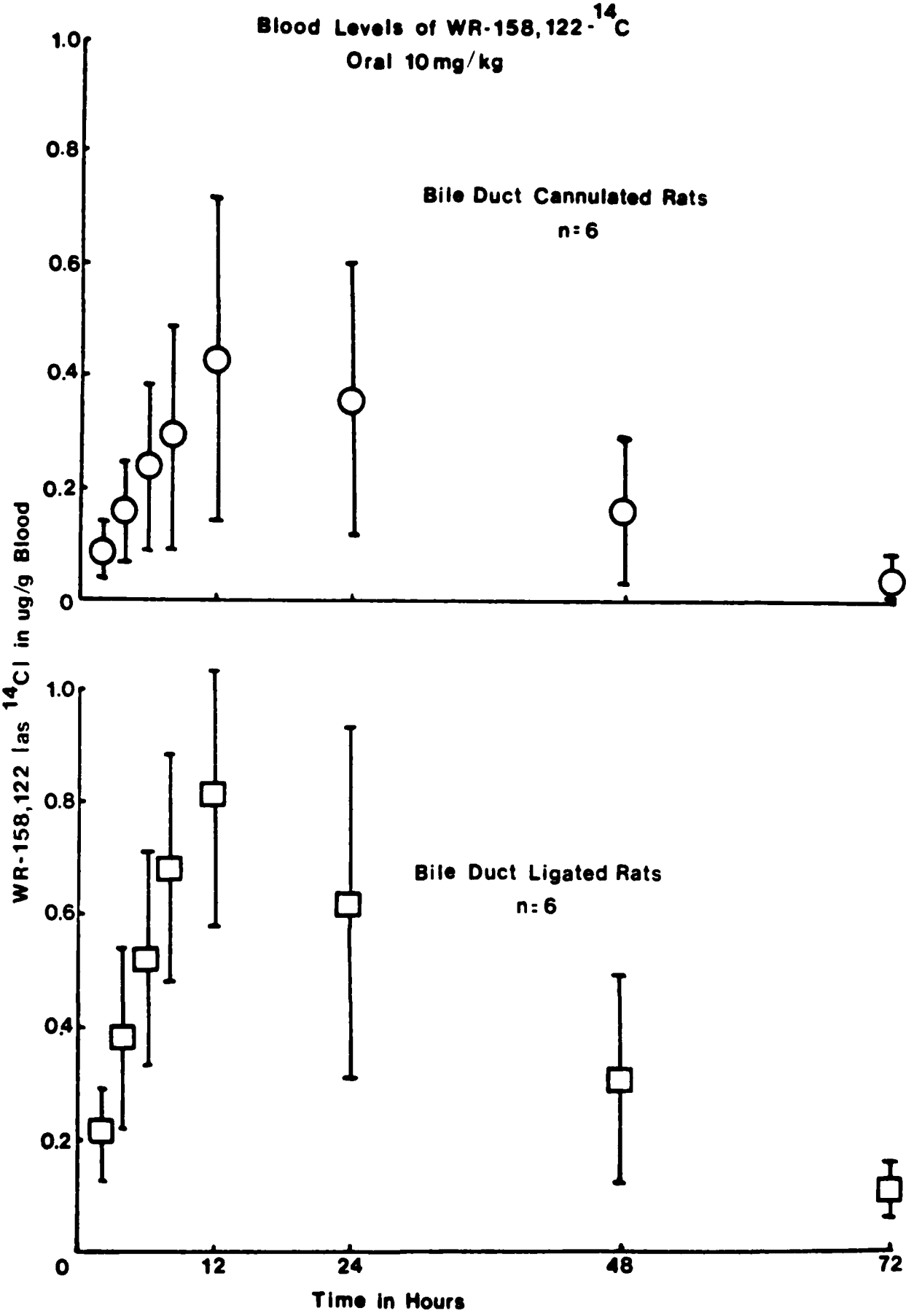

Blood Levels of WR-158,122-^{14}C
Oral 10 mg/kg
Bile Duct Cannulated Rats
n=6
Bile Duct Ligated Rats
n=6
WR-158,122 las ^{14}Cl in ug/g Blood
1.0
0.8
0.6
0.4
0.2
0
12
24
48
72
Time in Hours

Figure 1.2

Cumulative Excretion of WR-158,122

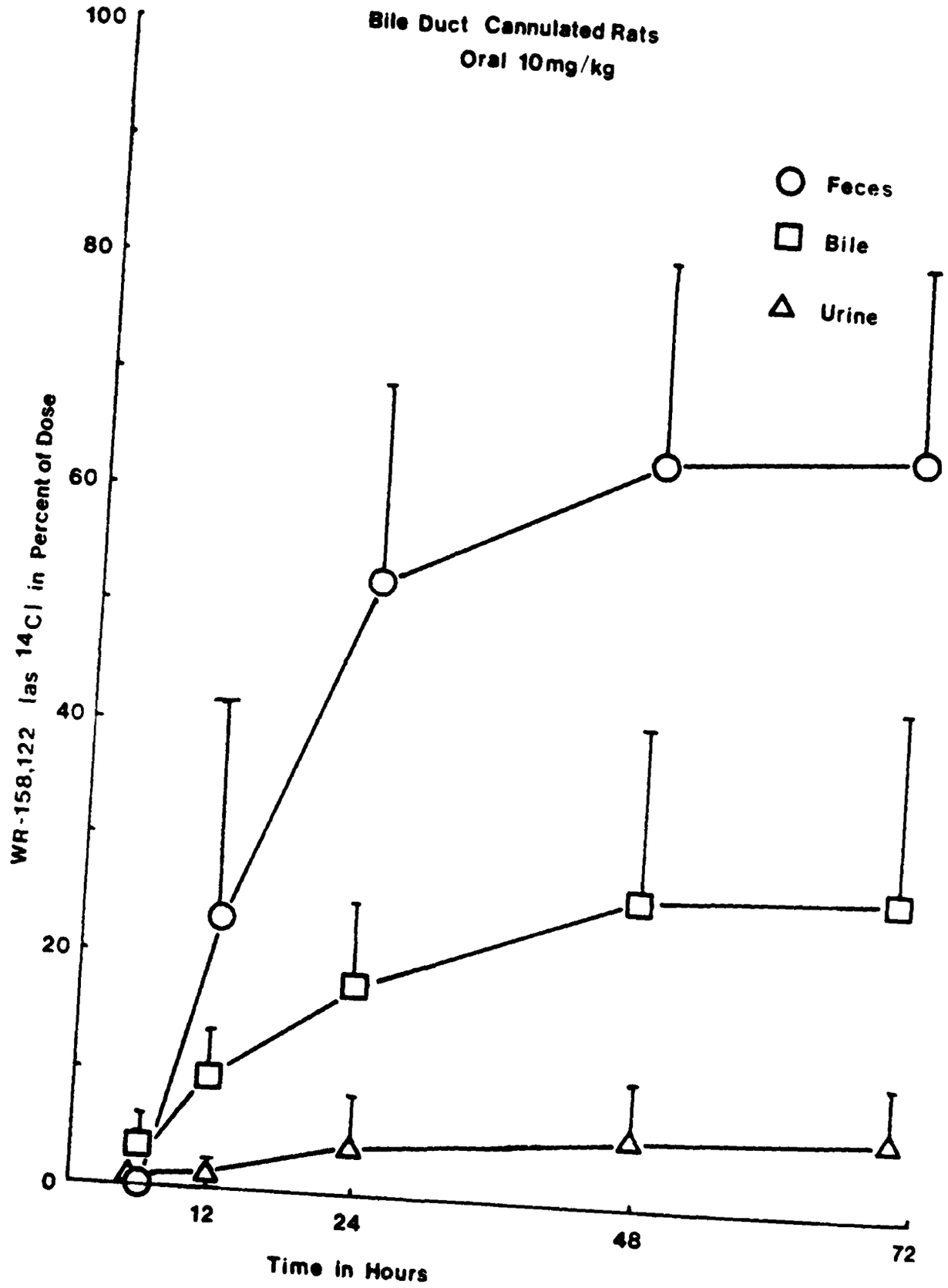
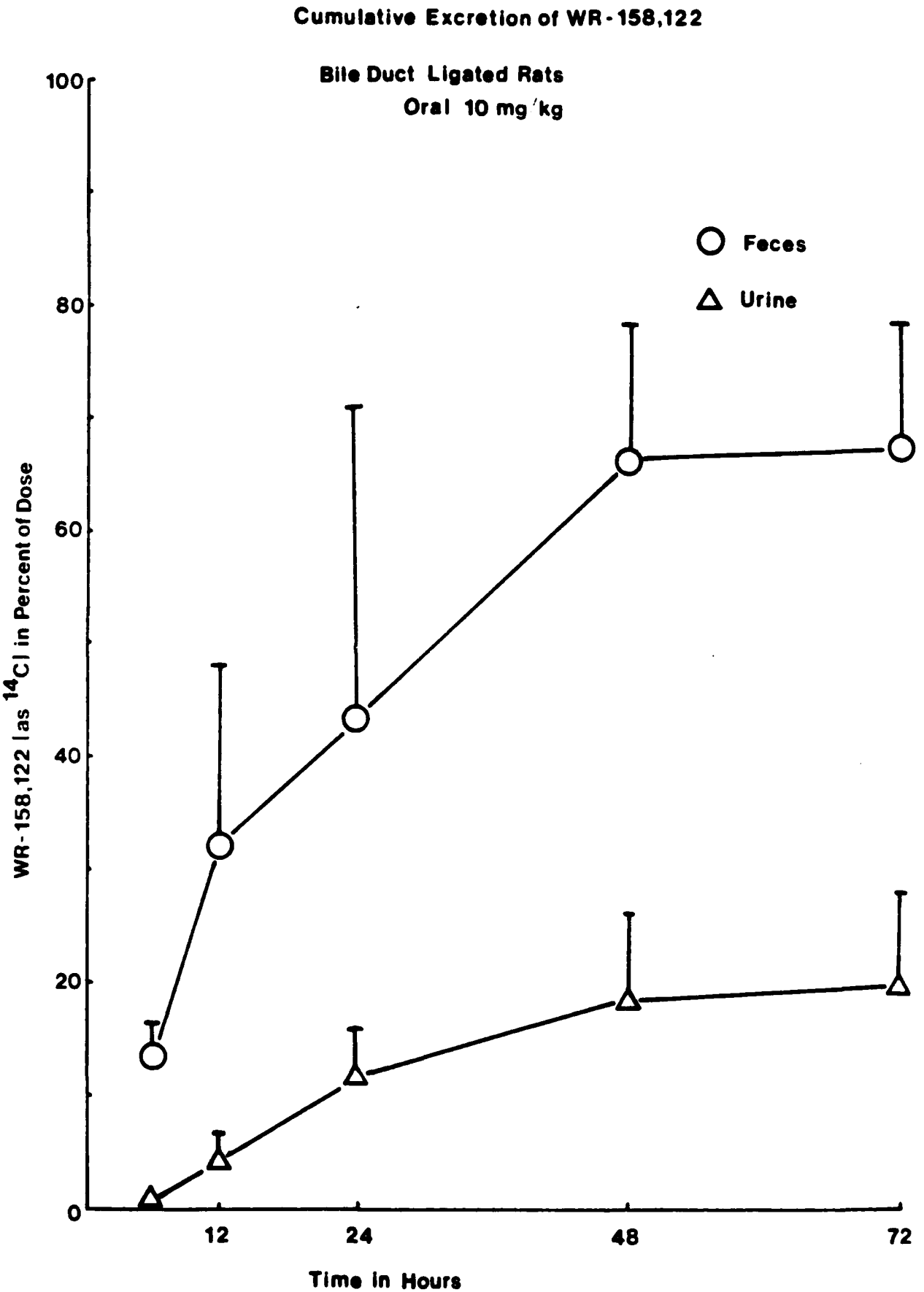
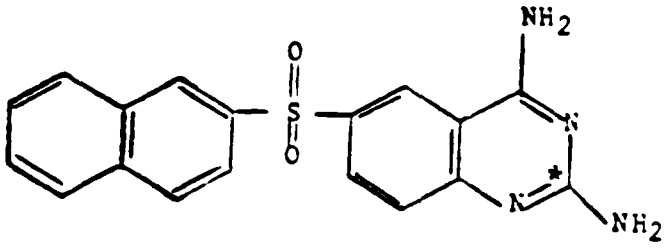


Figure 1.3


Cumulative Excretion of WR-158,122
Bile Duct Ligated Rats
Oral 10 mg/kg
Feces
Urine
WR-158,122 las ^{14}Cl in Percent of Dose
100
80
60
40
20
0
12
24
48
72
Time in Hours

SECTION 2. METABOLIC DISPOSITION OF LABELED WR-158,122 IN A BILE DUCT CANNULATED RHESUS MONKEY

Studies on the metabolic fate of WR-158,122 [2,4-diamino-6-(2 naphthylsulfonyl)-quinazoline-2-^{14}C], the antifolate antimalarial compound shown below



$*^{14}C$

have been carried out in one bile duct cannulated rhesus monkey. Data on blood levels, plasma levels, and excretion in bile, urine, and feces are detailed in this report.

2.2 MATERIALS AND METHODS

Compounds. WR-158,122 [2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline], empirical formula $C_{18}H_{14}SO_2N_4$, mol. wt. 350, was supplied by Walter Reed as Bottle AY 65858. The ^{14}C-preparation labeled in the 2 position was synthesized by Research Triangle Institute and was supplied as Lot No. 2572-110, dated 8/20/79, with a specific activity of 69 μCi/mg or 24 mCi/m mole. The radiochemical purity in a number of TLC systems was > 98%.

Treatment Suspension. The treatment suspension was prepared by grinding intimately 307.13 mg of cold drug and 3.08 mg of ^{14}C labeled drug in a glass mortar with a pestle, with small additions of diluent (0.2% methylcellulose and 0.4% Tween 80 in distilled water), until a smooth suspension was achieved. The suspension was decanted into a tared round-bottom polycarbonate 200 ml centrifuge bottle containing glass beads and diluted to 124.08 g. It contained 2.50 mg WR-158,122/ml and 1.86 μCi/ml. The suspension was stored at 4°C. Assay of the suspension gave the following results:

4,136,000 dpm/ml
1654 dpm/μg
1.86 μCi/ml

Analytical Procedures. Samples of blood, bile, urine and feces were collected as described in Interim Report No. 1 (November 20, 1979) and processed for liquid scintillation counting by the standard procedures of this laboratory as described in the same report.

Monkey. One 4 kg female rhesus monkey which had been acclimated to a primate chair was employed for this study. Water was available ad libitum

and the chair facilitated the collection of blood samples, collection of iced urine, feces, and bile.

The monkey's bile duct was cannulated on 12/4/79. The monkey was fasted for 24 hours prior to surgery. It was tranquilized with 0.25 ml Ketamine HCl, im, anesthetized with 1.5 ml sodium thiamylal, iv, and anesthesia maintained with methoxyfluorane.

A midline incision was used to expose the gall bladder and the common bile duct. A two centimeter length of latex tubing was double ligated over the common bile duct. A Bard latex T-tube (size 8 or 10) was installed into the fundus of the gall bladder. The exteriorized Bard T-tube was elongated by attaching a size No. 8 feeding tube. This tube delivered bile into a condom which was supported on a part of the monkey chair. The monkey could move freely without putting strain on the exteriorized cannula.

A second Bard T-tube (size 8 or 10) implanted in the duodenum permitted the administration of bile, liquid nutriments, or water.

On 12/8/79 the monkey escaped from the chair and pulled out the bile cannula. Corrective surgery was immediately performed and the bile cannula was re-installed, after which the monkey's recovery was uneventful.

On 12/11/79 the monkey was administered a single oral dose of WR-158,122 ^{14}C (5 mg/kg) via a No. 8 French nasogastric tube. The monkey vomited two times within five hours after this treatment so that a substantial portion of the dose was lost. Blood samples were collected from the right or left antecubital vein at 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours. Bile, urine and feces samples were collected at approximately the same times, as indicated in the tables. Each bile sample was weighed, ca 25% frozen for analysis and the remainder returned to the monkey.

On the 9th day following the first treatment, this monkey was given a second dose of WR-158,122 ^{14}C (5 mg/kg) by soft oral catheter. This dose was retained. Blood, bile, urine and feces samples were collected for the same time intervals as was done following the first treatment.

Two days after the second dose the monkey developed small decubitus ulcers on each elbow. For six days the monkey received 1 ml injections of Diathal®*, im, in addition to topical application of BFI®** powder and nitrofurazone*** ointment.

SMA 12/60 assays were run on plasma samples at 1 day after surgery, just prior to the first and second drug treatments and on the seventh day after the second treatment.

2.3 RESULTS

Blood Levels. Study of blood and plasma levels of radioactivity for the first treatment period shows that the peak levels occurred at about 8 hr. Detectable levels of the drug as ^{14}C in blood and plasma persisted through 72 hr(see Table 1). There was a marked decline in hematocrit levels from 24 hr (41%) through 72 hr (33%).

* Diathal® - Each ml contains 200,000 units procaine penicillin G, 250 mg dihydrostreptomycin, 10 mg chlorpheniramine maleate, and 25 mg diphemanil-methylsulfate. Preservatives are 20 mg procaine HCl, 8.8 mg sodium citrate, 10 mg lecithin (with 2% tricalcium phosphate) 1.5 mg methyl paraben and 0.5 mg propyl paraben as preservative. Water for injection q.s. Schering Corp., Kenilworth, NJ 07033.

** BFI® powder - Antiseptic first-aid powder. Contains Bismuth-formic-iodide, Zinc phenolsulfonate, Bismuth Subgallate, Amol (mono-n-amyl hydroquinone ether), Potassium Alum, Boric Acid, Menthol, Eucalyptol, Thymol and inert diluents. Calgon Consumer Products Company, Inc., Subsidiary of Merck & Co., Inc., Pittsburgh, PA 15230.

*** Nitrofurazone Ointment, N.F. - Topical Antibacterial Ointment (for external use only, not for ophthalmic use) distributed by Henry Schein, Inc., Port Washington, NY 11050.

Following the second treatment the peak for blood and plasma levels occurred at about 4 hr. Again the decline in hematocrit levels from 2 hr (37%) to 72 hr (29%) was marked.

Bile Excretion. Excretion of bile following the first treatment was not as abundant as that which followed the second treatment (see Table 2). During the 24 hours following the first treatment only 20.5 g of bile were excreted, whereas in the 24 hr following the second treatment 60.1 g of bile were excreted. Peak (2.2%) bile excretion occurred during the 48-72 hr interval following the first treatment, whereas the peak (7.8%) for the second treatment was a broad peak covering 0-12 hr.

From 24 to 48 hr following the first treatment only 19.2 g of bile were excreted. In contrast, during the same period following the second treatment, 88 g of bile were excreted.

The two vomiting episodes which occurred at 4 and 5 hours after the first treatment obviously reduced the amount of drug available for absorption and the delayed excretion pattern after the first dose may have been related to these.

Total radioactivity in the bile withheld (ca 25% of the total) amounted to 1.09% in first treatment and 2.63% for the second. The bile was not returned on a strict schedule. Frequently it was administered quite some time after the monkey's morning feeding. The bile samples were usually dark green and of low viscosity.

When the monkey was not eating satisfactorily on the second day of the first treatment period, a liquid nutriment called Esbilac® was administered

*Esbilac® - Skim milk, water, vegetable oil, casein, egg yolk, calcium carbonate precipitated, potassium phosphate monobasic, lecithin, calcium hydroxide, choline chloride, sodium bicarbonate, potassium chloride, carrageenan, salt, potassium phosphate, dibasic, magnesium carbonate, magnesium sulfate, vitamin A and vitamin E supplement, iron sulfate, zinc sulfate, niacin supplement, calcium pantothenate, copper sulfate, vitamin B_{12} supplement, vitamin D_3 supplement, manganese sulfate, riboflavin, thiamin HCl, pyridoxine HCl, potassium iodide. Borden Chemical, Borden Inc., P. O. Box 419, Norfolk, VA 23501 and Borden International, 420 Lexington Avenue, New York, NY 10017.

in two 20 ml infusions by duodenal cannula.

Urinary and Fecal Excretion. Following the first treatment the monkey excreted 13.1% of the dose in urine and 32.8% in feces giving a combined urinary and fecal excretion of 45.9% (as ^{14}C) (see Table 3). The peak for urinary excretion following this treatment was 7.6% of the dose during the 48-72 hr period. The peak for fecal excretion was 20.1% and occurred during the 24-48 hr period.

Following the second treatment the monkey excreted 21.5% of the dose in urine and 72.8% of the dose in the feces. Combined urinary and fecal excretion totaled 94.3%. It is noteworthy that 82% of the total urinary excretion was excreted during the 0-12 hr period.

The monkey was active and exhibited generally a good appetite for food (monkey chow with some fruit supplements) and water. The antibiotic therapy for six days did not appear to complicate the second study. Bile flow, fecal excretion and urine excretion were very good throughout the treatment period and for the following week.

The SMA 12/60 assays showed moderately elevated SGOT and LDH and appreciably elevated alkaline phophatase values. We had similar observations in a number of bile duct cannulated monkeys studied earlier (1969-1972).

2.4 DISCUSSION

The analysis of the first treatment of this bile duct cannulated monkey was seriously impaired by the vomiting episodes at 4 and 5 hr after dosing. From the total recovery of radioactivity in bile, urine, and feces (47%; Table 3) we concluded that about 50% of the dose was lost. The differences in the blood level curves and urinary excretion patterns suggested that absorption of the retained portion of the first dose was delayed.

When blood levels after the second treatment are compared to blood levels we observed previously in normal monkeys given the same dose of

WR-158,122^{14}C we find that the 2, 4, and 6 hr levels are very close to the mean blood levels of the normal monkeys (C. C. Smith et al., Interim Report No. 40-1, March 16, 1972; Contract No. DADA 17-67-C-7065). The 8, 12, 24, 48, and 72 hr blood levels after the second treatment are appreciably lower than the mean values for these same time periods in these normal monkeys. However, when the range for the 6 normal monkeys is considered, the levels we observed for these time periods in this bile duct cannulated monkey are close to the low figures for normal monkeys.

As in the studies of the 6 normal monkeys we find that plasma levels in the initial 2-24 hr period are appreciably higher than levels in whole blood. After 24 hr plasma levels are somewhat lower than whole blood levels.

The total excretion of 9.4% in bile during the second treatment indicates that this compound was moderately absorbed and to a limited degree excreted via the bile and urine. Eighty-three percent of the total radioactivity excreted in the bile (second treatment) was excreted by 12 hr. Biliary excretion beyond this time was relatively insignificant.

Further evidence that maximum absorption of the compound occurred in the 0-12 hr period (second dose) is the finding that 82% of the 21.5% excreted in the urine also was excreted in 0-12 hr. Only 3.9% of the dose was excreted in the 24-144 hr time period.

Urinary excretion in this bile duct cannulated monkey was higher than the mean observed for 6 normal monkeys (ibid), but almost identical urinary excretion was observed in one of the normal monkeys.

Most of the drug was excreted in the feces (i.e. 72.8%; second treatment) and primarily during the 24-48 hr period. Excretion via this route was practically complete by 72 hr. In our earlier studies of normal monkeys, the mean fecal excretion in 4 of the 6 normal animals accounted for 70.1% of the dose. Thus, fecal excretion in this bile duct cannulated monkey compared

favorably with observations on 4 normal rhesus monkeys studied seven years earlier.

Thus, from these limited data it appears that this antimalarial compound is moderately absorbed and to a limited degree excreted via the bile.

2.5 SUMMARY

1. A 4 kg female rhesus monkey with a bile duct cannula was administered two single oral doses of ^{14}C-labeled WR-158,122. The first dose was given 7 days after initial surgery and the second dose 9 days later. Part (approximately 50%) of the first dose was lost by vomiting but the entire second oral dose was retained.

2. Following the first treatment total excretion of drug in the urine (13.1%) and feces (32.8%) was equivalent (as ^{14}C) to 45.9% of the dose, not corrected for loss from vomiting. An additional 4.12% was excreted in the bile of which about 75% was returned to the animal.

3. Following the second 5 mg/kg oral dose, excretion (as ^{14}C) in the urine (21.5%) and feces (72.8%) accounted for 94.3% of the dose. An additional 9.4% was excreted in the bile and again about 75% was returned.

4. These data indicate that at least in this monkey, WR-158,122 (as ^{14}C) is moderately absorbed and to a limited degree excreted via the bile.

Table 2.1

Blood and Plasma Levels of WR-158,122 (as ^{14}C) in a Bile Duct Cannulated Monkey

Single Oral Doses of 5 mg/kg

Hours Post Dose	First Treatment* µg/g (as ^{14}C) Blood	Plasma	Hct	Second Treatment µg/g (as ^{14}C) Blood	Plasma	Hct
2	0.04	0.06	40	1.41	2.24	37
4	0.05	0.08	36	1.91	2.60	31
6	0.04	0.07	35	0.83	1.23	33
8	0.06	0.10	41	0.45	0.70	32
12	0.04	0.06	40	0.18	0.27	32
24	0.05	0.09	41	0.06	0.08	32
48	**	0.05	39	0.02	0.01	27
72	0.03	0.04	33	0.01	<0.01	29

* Vomited part of dose.

** Contaminated sample.

Table 2.2

Biliary Excretion of WR-158,122 (as ^{14}C) in a Bile Duct Cannulated Monkey

Single Oral Doses of 5 mg/kg

First Treatment*				Second Treatment			
Hours Post Dose	Percent Dose Period	Percent Dose Total	Wt. in grams	Hours Post Dose	Percent Dose Period	Percent Dose Total	Wt. in grams
0-6	0.04	0.04	2.6	0-6	3.9	3.9	14.2
6-12	0.36	0.40	15.4	6-12	3.9	7.8	21.4
12-24	0.17	0.57	2.5	12-24	0.91	8.71	24.5
24-48	1.2	1.77	19.2	24-48	0.55	9.26	88.0
48-72	2.2	3.97	38.8	48-72	0.07	9.33	63.4
72-96	0.11	4.08	48.8	72-96	0.02	9.35	73.0
96-120	0.02	4.10	27.9	96-120	0.01	9.36	75.0
120-144	0.01	4.11	26.3	120-144	<0.01	9.36	51.7
144-168	0.01	4.12	57.9	144-168	<0.01	9.36	76.0

*Vomited part of dose.

Table 2.3

Cumulative Excretion of WR-158,122 (as ^{14}C) in Bile, Urine and Feces from a Bile Duct Cannulated Monkey

Single Oral Doses of 5 mg/kg

First Treatment**

Excretion in Percent of Dose

Hours Post Dose	Bile* Period	Bile* Total	Urine Period	Urine Total	Feces Period	Feces Total	Combined Total
0-12	0.12	0.12	0.80	0.80			0.92
12-24	0.17	0.29	0.86	1.7	0.20	0.20	2.2
24-48	0.30	0.59	3.1	4.8	20.1	20.3	25.7
48-72	0.48	1.07	7.6	12.4	3.9	24.2	37.7
72-96	0.02	1.09	0.48	12.9	7.2	31.4	45.4
96-120	<0.01	1.09	0.13	13.0	0.92	32.3	46.4
120-144	<0.01	1.09	0.05	13.1	0.46	32.8	47.0
144-168	<0.01	1.09	0.04	13.1	N.A.***	32.8	47.0

Second Treatment

0-12	2.3	2.3	17.6	17.6			19.9
12-24	0.21	2.5	1.1	18.7	6.5	6.5	27.7
24-48	0.11	2.6	2.1	20.8	56.1	62.6	86.0
48-72	0.01	2.6	0.36	21.2	5.9	68.5	92.3
72-96	<0.01	2.6	0.13	21.3	3.7	72.2	96.1
96-120	<0.01	2.6	0.06	21.4	0.25	72.5	96.5
120-144	<0.01	2.6	0.07	21.5	0.32	72.8	96.9
144-168	<0.01	2.6	<0.01	21.5	<0.01	72.8	96.9

* Figures represent only portion saved for analysis
** Vomited part of dose
***Not assayed

SECTION 3. EXTRACTION OF LABELED WR-158,122 FROM RAT AND MONKEY URINE AND BILE. INITIAL STUDIES.

In our first interim report (11/20/79) we described the data we obtained on the absorption, excretion and distribution of ^{14}C following single oral doses of WR-158,122-^{14}C in control, bile duct ligated (BiDuLi) and bile duct cannulated (BiDuCa) rats. The second report (1/18/80) summarized data on blood levels and excretion of WR-158,122 in a BiDuCa monkey following single oral doses. In this report we are presenting our initial findings in developing extraction procedures for separating and isolating WR-158,122 and its metabolites from urine and bile using the samples obtained in the course of our previous studies.

The general approach consists of extracting biological samples with a series of solvents with varying solubility parameters (see appendix). Because some of the desirable solvents are miscible with water, a salting out procedure was used to provide, whenever possible, separate solvent and aqueous phases.

3.2 MATERIALS AND METHODS

These initial studies were carried out on urine and bile from control (untreated) rats and monkeys and treatment urine and/or bile from BiDuLi and BiDuCa rats and a BiDuCa monkey.

The ^{14}C standard used to spike control monkey and rat urine and monkey bile was prepared as follows. One ml of the treatment solution used in Interim Report No. 2 was diluted to 10 ml with DMSO. One ml of this standard was diluted to 25 ml with control urine or bile giving spiked samples which contained about 17,000 dpm of ^{14}C-WR-158,122 per ml.

The general protocol used in these initial extraction studies is described, for convenience, at the bottom of the first table and was the same in all the experiments. We have concentrated our attention on organic solvents that

form two phases after the addition of KBr, a neutral salt, to the urine or bile. The procedure appears to work well and so far has not been plagued by formation of emulsions.

3.3 RESULTS

In Table 1 one observes that all or almost all the ^{14}C in the urine was present in the solvent phase in those solvents (n-propanol, dimethylformamide, pyridine and methanol) which formed only one phase (and salt).

However, the solubility of parent drug and/or ^{14}C-containing metabolites in the solvents forming 2 phases was quite variable. EDC-2-ethyl hexanol (8:2) (EDC-2EH), a solvent mixture more polar than EDC (alone), benzene, ether, chloroform or CCl_4, appeared to remove only about 8.3% of the total urinary ^{14}C. This fraction probably consists of parent drug and possibly a relatively nonpolar metabolite(s). More polar solvents such as ethyl acetate, acetonitrile (ACN) or tetrahydrofuran (THF) extracted increasing quantities of urinary metabolites.

The results in Table 2 show that one can quantitatively extract the parent compound from spiked control monkey urine with all of the two phase solvents and with a representative single phase solvent, n-propanol. Therefore, we would conclude that all of the parent drug in monkey treatment urine was extracted by all of the organic solvents shown in Table 1. In addition, there were increasing amounts of metabolites extracted by ACN and THF; the quantity extracted depending on the polarity of the solvent used. We assumed that all single phase solvents would function alike; therefore, after the first experiment we used only one single phase solvent, n-propanol. From its solubility parameter it should be the least polar of the single-phase solvents.

Application of the same extraction scheme to a monkey treatment bile

sample provided the results shown in Table 3. If all the parent drug is extracted by EDC-2EH (as one would assume,(see Table 4) then one must conclude that the amount of parent compound in this animal's bile at 72 hr is no more than about 3% of the total ^{14}C. Also the ^{14}C-containing metabolites in this sample must be quite polar since even THF extracted only about 27% of the total ^{14}C in the bile.

The data in Table 4 indicate that WR-158,122-^{14}C is quantitatively extracted from spiked control monkey bile. These results are essentially parallel to those in Table 2.

When the extraction procedure was applied to a 24 hr bile sample from a BiDuLi rat dosed with WR-158,122-^{14}C we obtained the results shown in Table 5. The EDC-2EH soluble fraction was 3.9%, ACN and THF extracted 14 and 17% respectively and as would be expected almost all of the ^{14}C was soluble in N-propanol. We have assumed that the amount of parent compound in the bile or urine would decrease with time after dosing and our limited data support this assumption.

When urine from a representative BiDuLi rat was extracted in the same fashion (Table 6) one notes that more ^{14}C was extracted by these solvents from the urine than from the bile suggesting either that lesser amounts of extractable metabolites are present in bile or that bile is excreting an unexplained repression of the transfer of ^{14}C-containing metabolites to the organic phases. This will be taken up in the discussion.

Urine from a rat with BiDuCa and treated with an oral dose of ^{14}C-WR-158, 122 gave an extraction profile (see Table 7) which was similar to that in Table 6 except for one point. EDC-2EH extracted more ^{14}C after BiDuCa and this is opposite from what we would have expected. However, since there was less ^{14}C excreted in the urine in BiDuCa rats perhaps a higher percentage may be

"parent-like" compounds (extractable with EDC-2EH).

Finally, control rodent urine spiked with WR-158,122-^{14}C gave the same results as were obtained with control monkey urine and bile indicating that WR-158,122 was completely extractable from urine or bile by any of the solvents we used.

3.4 DISCUSSION

These data suggest strongly that one can develop an extraction procedure for characterizing the metabolites of WR-158,122 in either bile or urine samples from both rats and rhesus monkeys. The solvents can be applied sequentially and thus would permit us, hopefully, to evolve a scheme for isolating individual metabolite fractions. These fractions can be further defined using TLC or HPLC and such experiments are now being planned.

The low recovery of ^{14}C activity from bile may be the result of absolute differences in amounts of WR-158,122 and metabolites present or reflect an undefined inhibiting effect of bile constituents on extraction. This ambiguity will be resolved in our next interim report.

Several other prelinimary extraction studies are also underway in which we will saturate urine or bile with an acidic salt such as $(NH_4)_2SO_4$ or basic salts such as K acetate (pH 9.7) or K_2CO_3 (pH 11.7). It will be interesting to compare these results with those obtained with KBr. We also need to explore more non-polar solvents so that we can improve the specificity of the initial extraction for parent drug.

3.5 SUMMARY

1. Urine and bile samples from control (untreated) rats and rhesus monkeys as well as from bile duct ligated (BiDuLi) rats and bile duct cannulated (BiDuCa) rats and monkeys treated with WR-158,122-^{14}C have been extracted with a series of organic solvents in an attempt to define the profile of biliary and urinary metabolites.

2. Extraction of a urine sample from a BiDuCa monkey demonstrated that when the urine was saturated with KBr very polar solvents such as n-propanol (NPRO), pyridine, dimethylformamide or methanol formed one phase (with some salt) and the solvent phase contained all or nearly all of the ^{14}C activity. Solvents forming 2 phases such as ethylene dichloride: 2-ethylhexanol (8:2 , EDC-2EH), ethyl acetate, acetonitrile (ACN) or tetrahydrofuran (THF) extracted increasing amounts of ^{14}C-containing metabolites ranging from 8.6% to 66.2%.

3. NPRO, EDC-2EH, ACN and THF extracted all or nearly all of the ^{14}C activity from control simian urine and bile and from control rat urine.

4. EDC-2EH, ACN and THF extracted increasing amounts of ^{14}C from a 72 hr monkey treatment bile but even THF extracted only 27% of the total ^{14}C content. The total parent drug in this sample was 3% or less.

5. The ^{14}C activity in a 12 hr treatment bile sample from a BiDuCa rat gave an extraction profile not much different from that obtained with simian treatment bile; less than 4% of the activity comprised parent drug.

6. When one compares 24 hr urine samples from BiDuCa and BiDuLi rats one finds that the only significant difference in the profiles is in the amount of ^{14}C activity extracted by EDC-2EH which was about 14% for the BiDuLi rat and 23% for the BiDuCa animal.

7. These data provide almost indisputable evidence that WR-158,122 is absorbed and metabolized in the albino rat and rhesus monkey and the proportion of extractable ^{14}C activity is greater in the urine than in the bile. This is especially true for the monkey.

Table 3.1

Extraction of WR-158,122 ^{14}C Treatment Urine* From A Bile Duct Cannulated Rhesus Monkey

Single Oral Dose 5 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery (%)
EDC-2-Ethylhexanol (8:2)	5.2	2	8.3
Ethyl acetate	5.1	2	24.2
Acetonitrile	5.0	2	45.6
Tetrahydrofuran	4.9	2	66.2
N-propanol	5.9	1	93.7
Dimethylformamide	5.9	1	94.2
Pyridine	5.8	1	95.4
Methanol	6.0	1	96.5

*48 hr treatment urine containing 12,400 dpm/ml

Protocol:

1. Dissolve 0.5g KBr in each 1 ml aliquot in a graduated screw cap centrifuge tube.
2. Add 5 ml solvent.
3. Shake for 20 min and centrifuge.
4. Remove organic phase after recording color and volume of each phase and salt, if any.

Table 3.2

Extraction of Control Monkey Urine
Spiked with WR-158,122 ^{14}C

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery (%)
EDC-2-Ethylhexanol (8:2)	5.1	2	96.9
Acetonitrile	5.4	2	99.8
Tetrahydrofuran	5.2	2	104.1
N-propanol	5.9	1	97.6

Protocol same as Table 1.

Table 3.3

Extraction of WR-158,122 Treatment Bile*
from a Bile Duct Cannulated Monkey
Single Oral Dose 5 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery (%)
EDC-2-Ethylhexanol (8:2)	5.1	2	3.0
Acetonitrile	5.1	2	18.6
Tetrahydrofuran	5.2	2	26.8
N-propanol	5.7	1	103.8

*72 hr treatment bile containing 15,500 dpm/ml

Protocol same as Table 1.

Table 3.4

Extraction of Control Monkey Bile
Spiked with WR-158,122 ^{14}C

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery(%)
EDC-2-Ethylhexanol(8:2)	5.1	2	95.7
Acetonitrile	5.1	2	92.4
Tetrahydrofuran	5.1	2	95.2
N-propanol	6.1	1	97.3

Protocol same as Table 1.

Table 3.5

Extraction of WR-158,122 Treatment Bile*
from a Bile Duct Cannulated Rat
Single Oral Dose 10 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery(%)
EDC-2-Ethylhexanol(8:2)	5.05	2	3.9
Acetonitrile	5.2	2	14.4
Tetrahydrofuran	5.1	2	16.9
N-propanol	5.7	1	87.1

*12 hr treatment bile containing 62,000 dpm/ml.

Protocol same as Table 1.

Table 3.6

Extraction of WR-158,122 ^{14}C Treatment Urine* From
A Bile Duct Ligated Rat
Single Oral Dose 10 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery(%)
EDC-2-Ethylhexanol(8:2)	5.3	2	13.7
Acetonitrile	5.2	2	30.9
Tetrahydrofuran	5.3	2	38.1
N-propanol	6.0	1	87.1

*24 hr treatment urine containing 27,100 dpm/ml.
Protocol same as Table 1.

Table 3.7

Extraction of WR-158,122 Treatment Urine*
from a Bile Duct Cannulated Rat
Single Oral Dose 10 mg/kg

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery(%)
EDC-2-Ethylhexanol(8:2)	5.0	2	23.3
Acetonitrile	5.2	2	30.2
Tetrahydrofuran	5.0	2	38.9
N-propanol	5.7	1	88.4

*24 hr treatment urine containing 13,200 dpm/ml.
Protocol same as Table 1.

Table 3.8

Extraction of Control Rat Urine
Spiked with WR-158,122 ^{14}C

Solvent	Solvent Volume (ml)	No. of phases	Organic Phase Recovery (%)
EDC-2-Ethylhexanol	5.2	2	99.7
Acetonitrile	5.3	2	95.3
Tetrahydrofuran	5.2	2	103.0
N-propanol	5.9	1	98.4

Protocol same as Table 1.

APPENDIX

Common Solvents Listed According to Increasing Solubility Parameter (1)

Solvent	Sol. Par. (2,3)	H_2 Bonding (2)	Boil. Point	S.G.	Sol. of H_2O in Solv. (%)	Mol. Weight	Dielectric Const. (4)	Dipole Debye μ (4)
n-pentane	7.0	low	36.1	0.62	0.01	72.15	1.844	0.00
n-hexane	7.3	low	68.7	0.66	0.01	86.17	1.890	0.08
diethyl ether	7.4	0.19	34.5	0.71	1.5	74.12	4.335	1.15
n-heptane	7.4	low	98.4	0.68	0.015	100.2	1.924	0
cyclohexane	8.2	low	80.7	0.78	0.01	84.16	2.023	0
methyl, n-hexyl ketone	8.4	med.	173.5	0.82	insol.	128.21	-	-
carbon tetrachloride	8.6	low	76.8	1.58	0.01	153.84	2.238	0.00
diethyl ketone	8.8	med.	101.7	0.81	∞	86.13	17.00	2.70
toluene	8.9	low	110.6	0.86	0.06	92.13	2.379	0.39
ethyl acetate	9.1	0.12	77.0	0.90	3.3	88.10	6.02	1.81
benzene	9.2	low	80.1	0.87	0.05	78.11	2.284	0
chloroform	9.3	low	61.1	1.48	0.07	119.39	4.806	1.15
methyl ethyl ketone	9.3	med.	79.5	0.80	87.4	72.10	18.51	2.747
chlorobenzene	9.5	0.02	131.7	1.10	0.05	112.56	5.621	1.56
ethylene dichloride	9.8	low	83.5	1.25	0.15	98.97	10.36	2.06
p-dioxane	9.9	0.14	101.3	1.02	∞	88.10	2.209	0.45
acetone	10.0	0.14	56.2	0.79	∞	58.09	20.70	2.72
isoamyl alcohol	10.0	high	132.0	0.80	2.67	88.15	14.7	1.82
tert.-butyl alcohol	10.6	high	82.4	0.78	∞	74.12	10.9	1.66
pyridine	10.7	0.27	115.3	0.98	∞	79.1	12.3	2.20
sec. butyl alcohol	10.8	high	99.5	0.80	ca 12	74.12	15.8	-

Solvent	Sol. Par. (2,3)	H_2 Bonding (2)	Boil. Point	S.G.	Sol. of H_2O in Solv. (%)	Mol. Weight	Dielectric Const. (4)	Dipole Debye μ (4)
n-amyl alcohol	10.9	high	138.1	0.81	2.19	88.15	13.9	1.8
nitroethane	11.1	low	114	1.04	0.9	75.07	28.06	3.19
n-butyl alcohol	11.4	high	117.7	0.81	20.5	74.12	17.1	1.68
isopropyl alcohol	11.5	high	82.4	0.78	∞	60.09	18.3	1.68 (v)
acetonitrile	11.9	0.09	81.6	0.78	∞	41.05	37.5	3.37
n-propyl alcohol	11.9	high	97.2	0.80	∞	60.09	20.1	1.657
benzyl alcohol	12.1	high	205.5	1.04	ca 4	108.13	13.1	1.66
furfuryl alcohol	12.5	high	170	1.13	∞ (unstable)	98.1	-	1.92
ethyl alcohol	12.7	high	78.3	0.79	∞	46.09	24.30	1.68 (v)
methyl alcohol	14.5	0.28	64.5	0.79	∞	32.04	32.68	1.664
formamide	> 16.1	high	210.5	1.13	∞	45.04	109.5	3.37
glycerol	16.5	high	290	1.26	∞	92.09	42.5	2.56
water	23.4	high	100	1.0	-	18.02		

1. Solubility parameter $\delta = (\Delta E/v)1/2$ where ΔE is the energy of vaporization to a gas at zero pressure and v is the molal volume of the liquid (v = mol. wt./ density).

2. Burrell, H. Solubility Parameters, Interchemical Review, 14:3 (1955); low = < 0.08, high = 0.15 or >.

3. Hildebrand, J. and Scott, R. "The Solubility of Nonelectrolytes," New York: Reinhold Pub. Corp. 1950.

4. Weissberger, A. "Technique of Organic Chemistry" Vol VII, New York: Interscience Publishers.